LABORATORY COLONIZATION AND LIFE CYCLE OF *COQUILLETTIDIA CRASSIPES* IN MALAYSIA

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ABSTRACT. Methods are described for the laboratory colonization of *Coquillettidia crassipes*. The highest rate of insemination occurred in 60 × 60 × 120 cm cages and better insemination in laboratory adapted F_{15} generation. Embryonation and hatchability of eggs ranged from 69.6 to 97.9% and 63.3 to 94.3% respectively. Gravid females laid egg rafts on water in 500 ml beakers with small leaves of *Salvinia* for resting. Newly hatched larvae were set up in a basal medium of guinea pig dung and water or liver powder, yeast powder and water. Larvae attached to aquatic plants or 'Keaykolour' ruffia snow white paper. The cultures with paper gave better yields. At present 21 generations of *Cq. crassipes* have been reared in the laboratory.

INTRODUCTION

Coquillettidia crassipes (Van der Wulp) is an ornithophilic mosquito having a distribution from the Indian subcontinent through the Southeast Asian Region to Papua New Guinea and North Australia. In Sri Lanka, Niles et al. (1965) and Dissanaike and Fernando (1965) discovered that *Cq. crassipes* was the natural vector of *Cardiofilaria nilesi* in poultry. This finding was of considerable significance in that the prepatient period of the filarioid in poultry was as short as 21 days (Niles et al. 1965, Niles and Kulasiri 1970). The host-parasite combination was described as a promising model for filariasis research.

The main difficulty experienced in the study of *C. nilesi* was the lack of laboratory-colonized *Cq. crassipes*. Attempts to infect chickens with *C. nilesi* from Sri Lanka, in London using laboratory-bred *Aedes togoi* (Theobald) (Gooneratne 1969) were only partially successful. Later, Niles and Kulasiri (1970) used small numbers of laboratory-bred and wild caught *Cq. crassipes* to infect clean chickens. However, further studies on *Cardiofilaria* were abandoned due to the lack of colony material of *Cq. crassipes*.

In our studies on *Mansonia* in relation to the transmission of Brugian filariasis in Malaysia, natural infections of subperiodic *Brugia malayi* and *C. nilesi* were found in *Cq. crassipes* (Chiang et al. 1984, Mak et al. 1984, Chiang et al. 1986). Laboratory colonized *Cq. crassipes* is essential to study its susceptibility to subperiodic *B. malayi* and *C. nilesi*.

Three species of *Mansonia, Mansonia uniformis* (Theobald), *Ma. indiana* (Edwards) and *Ma. bonneae* (Edwards) have recently been colonized in the laboratory at the Institute for Medical Research, Malaysia (Chiang et al. 1985). The methods have been used with some modification to colonize *Cq. crassipes*.

MATERIALS AND METHODS

The habitats of *Cq. crassipes* overlap with those of *Mansonia*. The larvae of both genera attach themselves to the roots of aquatic plants with consequent difficulties in laboratory colonization.

The methods for maintenance of wild caught, blood-fed females till they became gravid, setting up of larval cultures using guinea pig dung and liver-yeast infusions with plants and paper[1] for larval attachment in indoor and outdoor insectaries, the insectary conditions, frequency of replacement of plants and paper, harvesting and maintenance of pupae were the same as used for *Mansonia* (Chiang et al. 1985).

Unlike the *Mansonia* species colonized, *Cq. crassipes* does not mate in 250 ml paper cups or small cages. Mating trials were conducted in cages of three different sizes, 30 cm cube, 60 cm cube and 60 × 60 × 120 cm. Males and females were introduced into the cages in the ratio of 2:1. Insemination was compared in the F_1 and the F_{15} generations.

Coquillettidia crassipes lays boat-shaped egg rafts rather like *Culex* but broader in the middle. Gravid females were introduced into 500 ml beakers with water and young *Salvinia* plants and covered with netting. Oviposition always occurred on the surface of water, not immediately after the females were introduced, as in *Mansonia*, but during the night. Samples of egg rafts, after the eggs hatched, were examined under the stereoscopic microscope for non-embryonated eggs.

RESULTS AND DISCUSSION

COLONIZATION. Table 1 shows the results of the mating trials in the paper cups and in the cages of different sizes. Insemination occurred in all three cage sizes, the highest rate being in the 60 × 60 × 120 cm cage. Better insemina-

[1] "Keaykolour" ruffia snow white paper (substance 250 GSM) manufactured by Wiggins Teape Paper, Ltd., Birmingham, U.K.

Table 1. Results of natural mating of *Coquillettidia crassipes* in different sizes of containers.

Container	Size of container	Generation of the mosquito	Ratio ♀ : ♂ used per container	No. of experiments	No. of female mosquitoes	
					Dissected	Insemination rate (%)
A	Paper cup (250 ml)	F_1	6 : 12	2	12	0
		F_{15}	6 : 12	3	17	0
B	Cage 30 × 30 × 30 cm	F_1	15 : 30	1	14	21.4
		F_{15}	15 : 30	2	28	25.0
C	Cage 60 × 60 × 60 cm	F_1	30 : 60	2	54	27.8
		F_{15}	30 : 60	2	57	54.4
D	Cage 120 × 60 × 60 cm	F_1	45 : 90	2	83	74.7
		F_{15}	45 : 90	2	87	93.1

* ♀♀ and ♂♂ were allowed to mate in the different containers for 5 days before ♀♀ were dissected and examined for insemination.

tion was observed in the laboratory adapted F_{15} generation in all three cage sizes.

Embryonation and hatchability of eggs of females mated in the 60 × 60 × 120 cm cage were monitored through 9 generations. Samples of 22, 16, 22, 18, 17, 16, 12, 7 and 9 egg rafts were examined during the successive generations. The mean egg counts ranged from 102.1 to 196.2. Embryonation and hatchability showed a range of 69.6–97.9% and 63.3–94.3%, respectively.

Table 2 gives the relative success of the cultures in the two insectaries. The best results were obtained with paper using guinea pig dung infusion at 28–30°C with yields of 43.5% pupation and 25.5% adult emergence. As in the case of *Mansonia,* the cultures with paper gave better overall yields. Only two types of plants were tested, *Eichhornia* which is the common aquatic plant in Malaysia associated with *Mansonia* and *Coquillettidia,* and *Alternanthera,* a weed associated with *Cq. crassipes* in Sri Lanka (Niles and Kulasiri 1970). *Eichhornia* proved unsuitable, the highest mean emergence at 28–30°C being 7.1%. *Alternanthera* gave a consistent emergence ranging from 10.2 to 11.8%.

Results from individual cultures showed roughly similar proportions of pupae emerging as adults from the two types of attachment sites, 50.9–58.9% from paper and 48.5–61.9% from plants. The comparable percentages of adult emergence from pupae with the paper and plant cultures suggest that in *Cq. crassipes,* as in *Mansonia,* gentle dislodging of the pupae attached to paper with a small soft paint brush did not increase their mortality.

The percentage pupation, emergence and the number of adults emerged in each of the first 9 generations and in the generations 16 and 17 are given in Table 3. Pupation and emergence were better in the later generations.

While the dung medium provided the best yields with paper at 28–30°C, there was no

Table 2. Percentage of pupation and emergence and number of pupae and adults from first instar larvae of *Coquillettidia crassipes* set up in different culture media with 250 larvae in each culture.

	Attachment host	No. of tests	% Pupation	(Range)	Mean % emergence	(Range)
Indoor Insectary (24–26°C)						
Guinea pig dung	Paper	29	26.6	(0.8–68.4)	14.5	(0.0–34.4)
	Eichhornia	5	10.8	(0.0–24.7)	5.7	(0.0–14.1)
	Alternanthera	5	19.1	(0.0–37.2)	10.2	(0.0–26.4)
Liver-yeast infusion	Paper	16	26.1	(1.6–50.8)	12.6	(1.2–28.4)
	Eichhornia	6	11.5	(0.0–18.5)	6.6	(0.0–12.1)
	Alternanthera	9	19.2	(0.0–49.2)	11.8	(0.0–34.8)
Outdoor Insectary (28–30°C)						
Guinea pig dung	Paper	34	43.5	(0.0–86.8)	25.5	(0.0–54.8)
	Eichhornia	5	11.9	(1.5–20.4)	7.1	(0.0–14.8)
Liver-yeast infusion	Paper	19	23.8	(0.0–53.2)	12.1	(0.0–50.8)
	Eichhornia	5	7.9	(0.0–18.5)	3.8	(0.0–10.6)
	Alternanthera	10	20.2	(0.0–46.4)	11.6	(0.0–23.2)

Table 3. Production of adults of *Coquillettidia crassipes* in the laboratory.

Generation	% pupation	No. of adults emerged		% emergence
		Males	Females	
F_1	30.7	1,467	1,439	16.5
F_2	31.2	207	205	18.3
F_3	57.8	202	183	33.2
F_4	22.7	126	109	11.3
F_5	39.2	248	237	23.9
F_6	32.1	351	310	20.9
F_7	24.0	244	189	15.5
F_8	22.0	223	168	12.4
F_9	30.6	133	131	20.5
F_{16}	62.0	640	712	38.6
F_{17}	68.8	506	485	36.0

difference between the emergences from the other cultures with dung and liver yeast media. The results suggest that the standardized liver yeast medium and paper can be used to colonize *Cq. crassipes.*

LIFE CYCLE. Maximum synchrony of pupation, i.e., minimum time for the entire population's pupal ecdyses, is achieved with the right balance of larval diet that would not contaminate the culture at optimum temperature and with the correct number of larvae in each culture dish. These factors were not studied in detail. However, in the more successful cultures using paper, the duration of larval development, pupation and adult emergence were recorded.

At 28–30°C under photoperiod LD 12:12, the mean duration from oviposition to egg hatching was 2.66 days (range 2.46–2.96 days). The eggs hatched between sunset and sunrise. The observations made for *Cq. crassipes* are similar to those for *Mansonia.*

Pupation in indoor and outdoor insectaries occurred in 27.0 ± 2.89 days, respectively, in guinea pig dung infusion cultures in 26.9 ± 2.47 days and 22.9 ± 3.65 days, in liver yeast cultures. Eighty percent of the pupation is completed in 12 days in both insectaries. The duration of the pupal stage was 3 days. As in *Mansonia,* males emerged earlier than females in both insectary colonies.

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